The effect of nectar guides on pollinator preference: experimental studies with a montane herb

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Summary. Rare "albino" morphs of the montane larkspur *Delphinium nelsonii* differ from common blue-flowered morphs in overall flower color, and in the strength of a contrasting color pattern at the center of the flower that presumably guides pollinators to concealed nectar. Previous studies showed that bumblebees and hummingbirds discriminate against albinos when presented with mixtures of the 2 morphs, and that it takes these pollinators longer to fly between successive flowers on albino than on blue-flowered inflorescences. To explore the link between these observations, we measured pollinator preferences and flower-to-flower flight times ("handling times") before and after painting flowers in 2 alternative ways that enhanced albino nectar guides. In all of 16 experimental replicates discrimination against albinos was reduced or eliminated after painting, and albino handling times declined toward values for blue-flowered inflorescences. This consistent result indicates that an inferior nectar guide increases the energetic cost of foraging at albinos. Increased cost in turn explains discrimination, under the reasonable assumption that hummingbirds and bumblebees are sensitive to foraging economics.

Flowers of animal-pollinated plants are characterized not only by bright overall coloration, which serves as a long-distance advertisement, but often by patterns of contrasting color as well. C.K. Sprengel (1793) was the first to discuss color patterns within single flowers. He proposed that they are "nectar guides" (*Saftmale*) that direct pollinators to a concealed reward. Several modern studies have documented responses of flower visitors to color patterns consistent with this interpretation (e.g., Kugler 1943, Daumer 1958, Scora 1969, Jones and Rich 1972, Jones and Buchmann 1974).

The prevalence of nectar guides (e.g., Sprengel 1793, Kugler 1943, 1963, Daumer 1958, Kevan 1983) may reflect a common evolutionary response to pollinator preference for flowers whose rewards can be located and extracted efficiently (Waser 1983). In spite of the behavioral studies cited above, the hypothesis that nectar guides influence pollinator preference by increasing foraging efficiency has not been tested. For the past 10 years we have studied a polymorphism in overall flower color, and in color pattern, that lends itself to such a test.

Our study involved the small montane larkspur *Delphinium nelsonii* Greene (Ranunculaceae). The zygomorphic flowers of this perennial herb consist of 5 large petaloid sepals arranged around 4 central petals (Fig. 1). There are 2 small "guard" petals that cover the sexual parts, and 2 elongated "nectariferous" petals that form a nectar-containing spur in back of the flower. The anterior portions of the nectariferous petals extend to the face of the flower. In western Colorado most plants have dark blue or blue-purple sepals and guard petals that contrast markedly with the central nectariferous petals, which are white with a few fine blue lines. Rare plants, however – <0.1% of individuals in our populations – have pale or white sepals that do not contrast with petals in human-visible wavelengths. In such "albino" flowers and in normal blue ones, there is a contrast in near UV wavelengths, which petals absorb and sepals reflect. Thus both flower morphs have a color pattern that forms a target indicating the location of nectar (Waser and Price 1983; compare Sprengel 1793:278, Macior 1975, Laverty 1980, Kay et al. 1981 for other larkspurs). This putative nectar guide apears to be quantitatively superior in blue flowers, however: it exists over a greater range of wavelengths (human-visible as well as near UV) and thus covers a larger fraction of the visual spectra of nectar-foraging hummingbirds and bumblebees that pollinate *D. nelsonii* (e.g., Mazokhin-Porshnyakov 1969, Goldsmith 1980).

In previous papers (Waser and Price 1981, 1983) we documented that albinos set significantly fewer seeds than their blue-flowered neighbors and proposed that this selective disadvantage explains their rarity. Subsequent field and flight cage experiments suggested that albinos have low seed set because bumblebees and hummingbirds discriminate against them. Discrimination appears not to be a function of the length of time pollinators are exposed to albinos – we outline evidence supporting this assertion later – and occurs regardless of the relative frequency of albinos in an experimental mixture (Waser and Price 1983). There does appear to be an element of frequency-dependence to hummingbird visitation in the flight cage, but it is not strong, and there are no such effects in field experiments with birds or in any experiments with bees. Nor does discrimination correspond to any inferiority of nectar reward. On the other hand, we have noticed that it takes both bumblebees and hummingbirds longer to fly between successive flowers on albinos than on blue-flowered inflorescences. These longer flower-to-flower movement times – hereafter

"handling times" – should cause pollinators to experience a lower net rate of nectar intake on albinos. This is a sufficient reason for discrimination against albinos by foragers that are sensitive to the economics of extracting food from flowers, a point we return to later (see also Waser 1983).

It is appealing to propose that longer handling times on albinos have to do with inferior nectar guides. Indeed, we were able to reject the possibility that something other than color is involved – such as flower morphology or spacing along the inflorescence – and in one experiment with a hummingbird we found that both discrimination against albinos and the difference in handling times disappeared after we painted sepals and guard petals blue (Waser and Price 1983). This experiment did not allow us to distinguish the effect of the contrasting target on pollinator behavior from that of overall flower color, however, because by painting sepals and guard petals we changed overall color as well as target intensity. Overall color alone might explain discrimination if pollinators have an innate preference for blue, or if color dictates contrast of the flower with its background and this influences the probability that an inflorescence is detected and approached from a distance.

Here we report results of further experiments in which we painted either sepals and guard petals or nectariferous petals in an effort to separate effects of overall flower color from those of target contrast. The results are consistent with the view that a contrasting target influences pollinator foraging efficiency and hence preference for a particular floral morph. They also suggest a role of overall color, at least under our experimental conditions.

Methods

We studied foraging by introducing 2–4 queen bumblebees (*Bombus appositus, B. nevadensis*, or *B. flavifrons*) or a single hummingbird (male *Selasphorus platycercus*, or male or female *S. rufus*) into an outdoor flight cage containing 5 albino and 5 blue-flowered potted plants matched closely in size and flower number. Flower number per plant ranged from 2 to 18; total numbers in experimental replicates generally were between 50 and 80. Plants were separated by approximately 20 cm and were alternated in a line as follows:

A B B A A B ....

Thus all plants except those on the ends had a nearest neighbor of each color morph. Plants were positioned in front of a sunlit flight cage window so that flowers were backlit. We recorded flower visits, filmed them at 9 or 18 frames/sec, and counted film frames to measure flight times between successive flowers on single inflorescences.

In looking for handling time differences between color morphs we restricted attention to flights between flowers, which include the time taken for a pollinator to position itself at the face of the flower before inserting its mouthparts into the nectariferous spur. This is just the process that should be sensitive to nectar guide characteristics. Our definition of handling times is unusual (compare Harder 1983); in essence we are considering one component of the handling of an entire inflorescence rather than a single flower. Time spent at the flower is another component, but it is not obvious how it will depend on color variation.

To obtain a reasonable sample of visits to unmanipulated flowers we had to expose them to birds for about
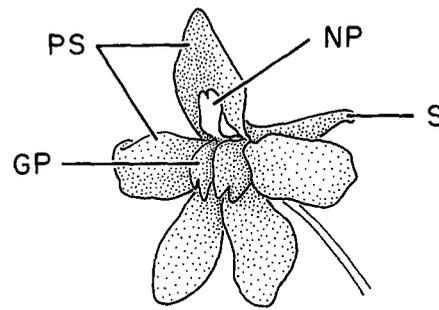

Fig. 1. Anterior view of a *D. nelsonii* flower. *PS* petaloid sepals, *NP* nectariferous petals, *GP* guard petals, *S* spur

1.5 h and to bees for about 3 h. In some experiments we ran 2 successive trials, separated by 2–46 h, with unmanipulated flowers. We then removed plants from the flight cage, painted them as described below, allowed them to accumulate nectar for 3–24 h, and reintroduced them to pollinators. Occasionally pollinators did not forage promptly; we then suspended the trial temporarily. Complete experiments with birds and bees lasted as long as 24 and 48 h, respectively. Individual birds were released after one experiment whereas bees were sometimes used for 2–3 experiments and kept chilled in between. Before starting any experiment we exposed the bird or bees involved to albino and blue "training" flowers augmented with a sucrose solution approximating the concentration of *D. nelsonii* nectar, and made sure they had fed from both color morphs.

We painted flowers in one of two ways, and always painted blue flowers along with albinos as a control. In *sepal-painting* experiments we painted the 5 petaloid sepals and 2 guard petals (Fig. 1) with artists' acrylic paints matched closely to the common blue-purple flower color. These paints absorb near UV (Waser and Price 1983), so we were creating a contrasting target on albino flowers in human-visible wavelengths and reducing or eliminating the normal UV target of all flowers. In *target-painting* experiments, we painted the anterior parts of the nectariferous petals that extend to the face of the flower, again matching the normal blue color. This treatment extinguished the contrasting target of blue flowers in the human-visible but enhanced that of albinos; there was no effect on the UV target of either color morph.

We conducted 3 sepal-painting experiments with hummingbirds, 1 in 1981 and 2 in 1982, and 3 experiments with bumblebees in 1982. We conducted 6 target-painting experiments with hummingbirds, 4 in 1983 and 2 in 1984. and 4 experiments with bumblebees in 1983. We were able to film a satisfactory sample of flower-to-flower movements (> 10 per color morph per painting treatment) in all sepal-painting and 4 target-painting experiments with birds. We did less well with bees, meeting or approaching this criterion in only 2 sepal-painting and one target-painting experiments.

Results

(1) Sepal-Painting Experiments

Albinos were discriminated against initially in all 3 replicate sepal-painting experiments with hummingbirds (Fig. 2). Compared to expectations based on relative availabilities of plants albinos were undervisited by 10%–19% (responses
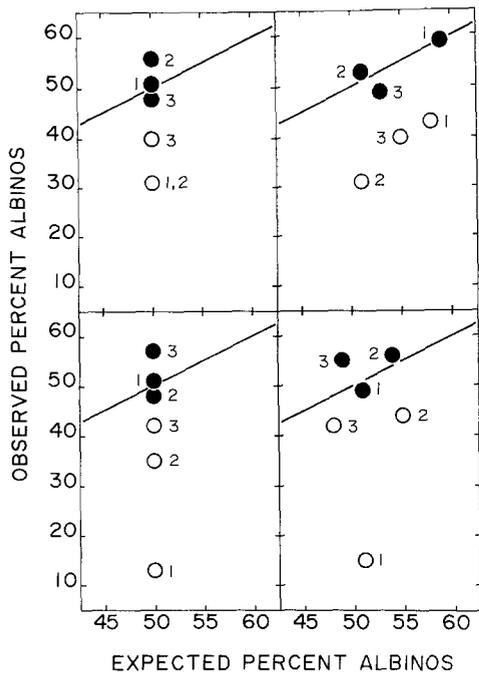
Fig. 2. Discrimination in sepal-painting experiments, for humming-birds (top) and bumblebees (bottom). Graphs on the left and right show choice based on plants and on flowers, respectively. Blue and albino plants were always presented in a 50:50 ratio; flower ratios often deviated slightly from this. These ratios determined "Expected Percent Albinos" values. Open and closed circles indicate choice before and after painting, respectively; each circle is identified by the number of the experimental replicate. The diagonal line is the line of no preference. Total numbers of visits across replicates were 736 to plants and 1,198 to flowers by birds, and 530 to plants and 1,292 to flowers by bees

Fig. 3. Discrimination in target-painting experiments. All conventions follow Fig. 2. Total numbers of visits across replicates were 979 to plants and 1,704 to flowers by birds, and 462 to plants and 951 to flowers by bees

based on flower availabilities were comparable and so will not be reported here or in the next section). Discrimination disappeared or was reversed slightly after painting; the response then ranged from 2% undervisitation to 6% over-visitation. Thus there was a shift toward the line of no preference in all experiments (Fig. 2). Handling times changed correspondingly. Albino handling times initially exceeded those on blue-flowered inflorescences by 17%–83%; after painting the situation was reversed slightly with albino handling times being 2%–20% shorter (Table 1).
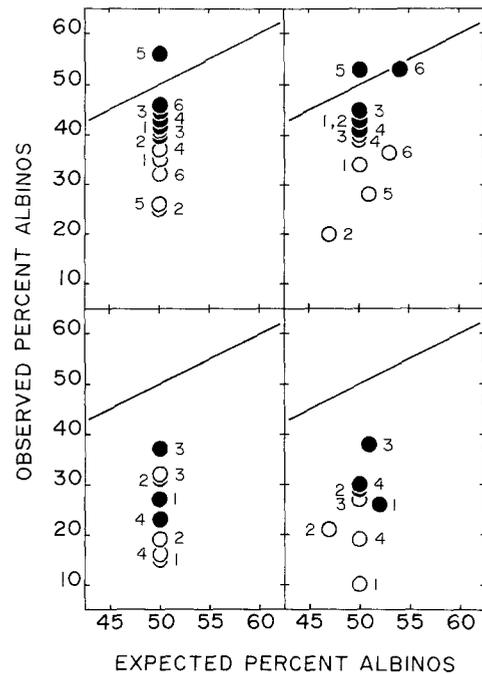
Discrimination by bumblebees paralleled that by hum-mingbirds (Fig. 2). In 3 replicate experiments albino plants were undervisited initially by 8%–37%; after painting the response ranged from 2% undervisitation to 7% overvisita-tion. Again albino handling times were longer initially, by 11%–22%; after painting they were 1% longer in one repli-cate and 5% shorter in the other for which we have reason-able samples (Table 1).

(2) Target-Painting Experiments

Target-painting results were similar to those just described (Fig. 3). Albino plants were undervisited initially by 10%–25% in all 6 replicates with hummingbirds. Discrimi-nation diminished after nectariferous petals were painted, although not as strongly as in sepal-painting experiments. After painting, the response ranged from 10% undervisita-

Table 1. Between-flower handling times, in seconds, from sepal-painting experiments. Values are means ± 1 se, with sample size (number of flights) in parentheses. Numbering of relicates corresponds to Fig. 2. Sample sizes in replicate no 1 for bumblebees were too small for analysis (see Methods section)

Replicate	Handling time			
	Before painting		After painting	
	Blue	Albino	Blue	Albino
Hummingbirds				
no 1, 1981	0.52±0.08 (16)	0.61±0.07 (25)	0.36±0.05 (14)	0.33±0.04 (30)
no 2, 1982	0.46±0.03 (24)	0.84±0.08 (20)	0.46±0.02 (15)	0.45±0.03 (16)
no 3, 1983	0.50±0.05 (14)	0.65±0.04 (23)	0.48±0.03 (10)	0.46±0.04 (18)
Bumblebees				
no 2, 1982	2.35±0.19 (19)	2.62±0.74 (22)	1.84±0.11 (15)	1.75±0.12 (27)
no 3, 1982	1.43±0.11 (13)	1.74±0.16 (13)	1.37±0.09 (7)	1.39±0.13 (8)

Table 2. Between-flower handling times from target-painting experiments. Conventions follow Table 1, and numbering of replicates corresponds to Fig. 3. Sample sizes in replicates no 3–4 for hummingbirds and no 2–4 for bumblebees were too small for analysis

Replicate	Handling time			
	Before painting		After painting	
	Blue	Albino	Blue	Albino
Hummingbirds				
no 1, 1983	0.42 ± 0.03 (30)	0.61 ± 0.05 (21)	0.53 ± 0.05 (23)	0.46 ± 0.04 (23)
no 2, 1983	0.48 ± 0.05 (17)	0.62 ± 0.05 (12)	0.47 ± 0.03 (18)	0.42 ± 0.03 (19)
no 5, 1984	0.81 ± 0.05 (20)	0.94 ± 0.07 (20)	0.57 ± 0.03 (29)	0.57 ± 0.04 (36)
no 6, 1984	0.49 ± 0.03 (23)	0.64 ± 0.04 (20)	0.52 ± 0.03 (38)	0.44 ± 0.05 (55)
Bumblebees				
no 1, 1983	1.66 ± 0.09 (32)	2.61 ± 0.44 (4)	1.81 ± 0.19 (12)	1.74 ± 0.23 (14)

tion to 6% overvisitation; slight undervisitation prevailed in 5 of 6 replicates. Handling times changed correspondingly. Albino handling times initially were 16%–45% longer; after painting they ranged from being equal to being 15% shorter (Table 2).

Albinos were also undervisited in all 4 replicates with bumblebees, by 18%–35% (Fig. 3). After painting, undervisitation was always reduced but never eliminated, unlike the situation with hummingbirds. It ranged from 13%–27%. Handling times changed correspondingly in the one replicate in which we obtained reasonable samples, being 57% longer before painting and 4% shorter after painting (Table 2).

Discussion

Our experimental results were strikingly consistent, even though they depended on the behavior of often-recalcitrant pollinators. Initial strong discrimination against albinos was eliminated after sepal painting in all of 6 experimental relicates ($P = 0.016$, 1-tailed sign test, Siegel 1956). Albino handling times declined towards, and converged on, values for blue-flowered plants in 5 of these for which we have reasonable samples ($P = 0.031$, 1-tailed). Discrimination was reduced after target painting in all of 9 replicates ($P < 0.002$, 1-tailed), and albino handling times converged on those for blue-flowered plants in 5 for which we have reasonable samples ($P = 0.031$, 1-tailed). Variation among replicates in the absolute magnitude of handling times (Tables 1 and 2) was usually slight. Some variation is expected, since we used several species of hummingbirds and bumblebees that vary in body size and morphology.

Because we used the same plants in unpainted and painted treatments within a replicate as a control for plant size and morphology, pollinators were always exposed to painted flowers last. Thus it is possible that preference and handling time changes were temporal rather than treatment effects. We can think of two reasons to expect a temporal effect. If foraging efficiency and choice depend on learning, discrimination might have declined along with increasing exposure to albinos in the flight cage, where their frequency and density were much higher than encountered in nature. In this case albino handling times should have declined as well. Another possibility is that initially undervisited albinos accumulated nectar rewards increasingly superior to those of blue flowers. Pollinators might have avoided them less strongly through time as a result. We can test for such

effects, because we often presented unpainted flowers in 2 trials separated by as long as one day. This feature is shared by a series of earlier flight cage experiments (Waser and Price 1983). A comparison of preferences in early and late trials with unpainted flowers from a total of 27 experiments with birds and bees reveals no consistent temporal change. Based on plant availabilities, discrimination against albinos decreased in 11 cases, increased in 12, and was unchanged in 4 ($P = 0.5$, 1-tailed sign test omitting "ties"). Similarly, we can compare albino handling times at the beginning and end of treatments with unpainted flowers. In this case we have values from 18 experiments; handling times decreased in 8 and increased in 10 ($P = 0.76$, 1-tailed). We conclude that experimental results were not due to the temporal sequence of treatments.

Instead, we attribute much of the treatment effect to manipulation of nectar guides. In sepal-painting experiments, pollinator discrimination disappeared in all cases after albino flowers were provided with a contrasting target in human-visible wavelengths. However, these experiments reproduced the common blue flower color in addition to providing a target, so they are not conclusive by themselves. Target-painting experiments did not share this drawback because only the small exposed portion of the central petals was painted. Since discrimination also declined uniformly following target painting, we conclude that flower choice is indeed related to the strength of the nectar guide.

The fact that discrimination declined less after target painting than after sepal painting suggests that overall color also may have played a role in choice. One possibility is that pollinators have an innate preference for blue. This explanation is contradicted by the similarity of hummingbird and bumblebee behavior, however, because it requires that these two distinct taxa have similar innate preferences that for some reason correspond to consistent handling time differences (see also Waser and Price 1981). This goes against parsimony as well as the common finding that hummingbirds are indiscriminate with respect to color (e.g., Goldsmith and Goldsmith 1979, Waser 1983 and references therein). Another possibility is that blue flowers contrast more strongly than albinos against the opaque white walls of the flight cage and thus render inflorescences slightly easier to locate from a distance. Such an effect seems unlikely to be repeated in nature, where the usual background is green foliage against which a white flower may well be more visible than a blue one (Kevan 1972, Mulligan and Kevan 1973).
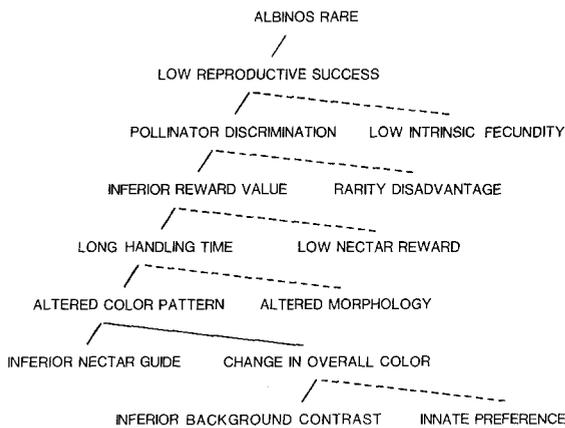
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                    ALBINOS RARE
                       /
              LOW REPRODUCTIVE SUCCESS
                     /¯¯¯¯¯¯¯¯¯¯¯¯¯¯¯
        POLLINATOR DISCRIMINATION    LOW INTRINSIC FECUNDITY
                  /¯¯¯¯¯¯¯¯¯¯¯¯¯¯
         INFERIOR REWARD VALUE      RARITY DISADVANTAGE
              /¯¯¯¯¯¯¯¯¯¯¯¯¯
      LONG HANDLING TIME      LOW NECTAR REWARD
            /¯¯¯¯¯¯¯¯¯¯¯¯
   ALTERED COLOR PATTERN    ALTERED MORPHOLOGY
         /¯¯¯¯¯¯¯¯¯¯¯¯¯¯
INFERIOR NECTAR GUIDE    CHANGE IN OVERALL COLOR
                      /¯¯¯¯¯¯¯¯¯¯¯¯¯¯¯
        INFERIOR BACKGROUND CONTRAST    INNATE PREFERENCE
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Fig. 4. The hierarchical relationship of succeedingly more detailed levels of explanation explored in our study of flower color. Observations at a level are alternative explanations for an observation at the level above; in some cases there may be other plausible explanations we have not considered. Experiments were used to distinguish among alternatives in most cases; solid lines link the alternatives receiving the most support. This paper deals with the bottom 2 levels of the hierarchy; upper levels are discussed in Waser and Price (1981, 1983)

Discrimination against unpainted albinos was always associated with handling times longer than those for blue inflorescences. This supports the idea that pollinator choice is sensitive to foraging economics, and specifically to net rate of reward intake. A previous statement to this effect (Waser and Price 1983) met with some skepticism, apparently because we observed partial preferences. Simple optimal diet models (e.g., MacArthur and Pianka 1966, Emlen 1966) do not predict such behavior, but they assume a fixed value of each diet item. Partial preferences are indeed predicted if the value of a diet item varies (Emlen 1968). Even if the expected value for albinos lies below that for blue flowers, some albinos could be rewarding enough to lie above the threshold for inclusion in the optimal diet. This argument depends on pollinators being able to assess some aspect of value that varies among individual plants. The most likely possibility in our system involves variation in the distance to a plant, and thus in the time and energy needed to reach it. It is easy to imagine that a pollinator leaving one plant might pick an albino next if it is sufficiently close; the range of acceptable distances from which albinos are approached might simply be shorter than the range for blue-flowered plants. This could allow pollinators to achieve some visitation rule that is optimal subject to the constraint of imperfect knowledge of other variable aspects of net value. We do not mean to imply that we can examine whether hummingbirds and bumblebees conform exactly to any particular optimality criterion. To do so we would have to estimate accurately the caloric reward obtained from each flower, among other things, which would require experiments we have not attempted (see Hodges and Wolf 1981).

The goal of our work over the last 10 years has been to determine in detail why albinos are rare in Colorado populations of *D. nelsonii*. Starting with the observation of rarity, we have concerned ourselves with a succession of further observations that form a "logical tree" (sensu Platt 1964). To explain an observation at any level of this hierarchical tree (Fig. 4), we formulated hypotheses at a level below; these subsequently became new observations in need of explanation. We have repeated this process to the point of understanding a great deal about how a floral trait influences pollinator behavior and how this feeds back on plant fitness. Indeed, carrying the investigation to this level was necessary to identify the trait of importance – a pattern of contrasting colors. While such a reductionist and mechanistic approach (sensu Price 1985) is time-consuming, we believe it avoids pitfalls of more superficial methods for exploring adaptation and holds great power for addressing questions of ultimate causation in evolutionary ecology.

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